IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of)
:
Yuki Katayama, et al.)
: Group Art Unit: 1657
Serial No. 10/531,315)
: Examiner: Amanda P. Wood
Filed: April 13, 2005)
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For: METHOD FOR QUANTITATIVELY DETERMINING CHOLESTEROL IN HIGH-DENSITY LIPOPROTEIN AND REAGENTS THEREFOR
)

DECLARATION

The Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Yuki Katayama of 905 SURPASS MISHIMAHONCHO, 1-10, Honcho, Mishima-shi, Sizuoka, Japan do declare as follows:

I finished my bachelor course of Agricultural Sciences, Faculty of Agriculture, Okayama University in March, 1995, and I was given the degree of B.A. I finished my master course at Graduate School of Agriculture, Okayama University in March, 1997, and I was given the degree of M.A.

Since April, 1997, I have been employed by KYOWA MEDEX CO., LTD.

Since April, 1997, I have been engaged in the research on development of diagnostic reagent kits, mainly related to lipids (e.g. HDL cholesterol).

In the Experiments shown below, I used the following reagents and enzymes.

HEPES (manufactured by BDH Laboratory), EMSE (manufactured by Daito Chemix Corporation), sodium dextran

sulfate (molecular weight: 500,000) (manufactured by Pharmacia), bovine serum albumin (BSA; manufactured by Oriental Yeast), Nymeen L207 (polyoxyethylene dodecylamine; manufactured by NOF), Triton X-100 [polyoxyethylene octylphenyl ether (HLB13.5); manufactured by Sigma], Nonion NS-230 [polyoxyethylene nonylphenyl ether (HLB17); manufactured by NOF], n-octyl-β-D-thioglucoside (manufactured by Dojindo Laboratories), n-heptyl-β-D-thioglucoside (manufactured by Dojindo Laboratories), 4-aminoantipyrine (manufactured by Saikyo Kasei), LPL6 (cholesterol esterase; manufactured by Amano enzyme), COO321 (cholesterol oxidase; manufactured by Toyobo).

Nonion NS-230 was used as an alternative of Emalex NPL-30 [polyoxyethylene nonylphenyl ether (HLB17)] in Miki et al.

Kits for quantitatively determining HDL cholesterol

Kits for quantitatively determining HDL cholesterol comprising the following first and second reagents were prepared.

First reagent

HEPES (pH7.5)	10 mmol/L
EMSE	0.3 g/L
sodium dextran sulfate (molecular weight: 500,000)	1.0 g/L
BSA	2.0 g/L
Detergent A	

Second reagent

HEPES (pH7.0)	10 mmol/L
4-Aminoantipyrine	0.3 g/L
Peroxidase	20 kU/L
LPL6	0.05 kU/L
COO321	3.0 kU/L

Table 1

Kits	Detergent A	concentration (g/L)
A	Nymeen L207	0.07
a	Triton X-100	0.1
b	Nonion NS-230	0.1
c	n-octyl-β-D-thioglucoside	0.1
d	n-heptyl-β-D-thioglucoside	0.1

Quantitative determination of HDL cholesterol

HDL cholesterol in 30 samples of human serum samples were measured on Hitachi 7170 autoanalyzer, using the Kit A and Kits a~d.

(1) Preparation of calibration curve

A calibration curve showing the relation between HDL cholesterol concentration and "absorbance" was prepared by the measurement on Hitachi 7170 autoanalyzer using a physiological brine (HDL cholesterol concentration: 0.0 mg/dL) and serum (HDL cholesterol concentration: 60.0 mg/dL) as standard solutions.

"Absorbance" used herein means a value obtained by subtracting E1 from E2 on the basis of the two absorbances (E1 and E2) measured in the following reaction.

A standard solution (3 mL) and the first reagent (0.24 mL) were added to a reaction cell and the mixture was heated at 37°C for 5 minutes. After measurement of absorbance (E1) of the reaction mixture at a main wavelength of 600 nm and a sub-wavelength of 700 nm, the second reagent (0.08 mL) was added to the reaction mixture and the mixture was heated at 37°C for 5 minutes. Absorbance (E2) of the last reaction mixture was measured at a main wavelength of 600 nm and a sub-wavelength of 700 nm.

(2) Calculation of "absorbance" for a human serum sample by the reaction of the sample with the Kit A

The same method as in the calculation of "absorbance" in (1) was carried out except that human serum sample was used instead of the standard solution used in the preparation of a calibration curve in (1) whereupon "absorbance" for the sample was calculated.

(3) Determination of HDL cholesterol concentration in a human serum sample

HDL cholesterol concentration in each sample was determined by correlating the "absorbance" calculated in (2) and the calibration curve prepared in (1).

The same operation as above was carried out except that each of the Kits a~d was used instead of the Kit A, whereupon HDL cholesterol concentration of each sample of the 30 human serum samples was determined.

In the meanwhile, HDL cholesterol concentration of each sample of the 30 human serum samples was determined according to a DCM (a Designated Comparison method) mentioned in Clinical Chemistry, vol. 45, No.10, p. 1803-1812 (1999), and the values to be measured were compared with those obtained by the

measurements using each of the Kit A and the Kits a~d.

Correlation coefficients between each of the values to be measured for each of the measurements and those for the DCM are shown in Table 2.

Table 2

Kits	Correlation Coefficient
A	0.98
a	0.57
b	0.70
c	0.57
d	0.59

As shown in Table 2, it was proved that the measurement using the Kit A comprising polyoxyethylene alkylamine (Nymeen L207), a surfactant of the amended claim 1, shows better correlation with measurement by DCM than measurement using the Kits a~d.

The undersigned declarant declares further that all statements made herein of his knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Executed this 23rd day of January , 2008.

Yuki Katayama

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